

WE CLAIM:

-1-

5 A transgenic plant which degrades lignocellulose when the transgenic plant is ground to produce a plant material comprising:

10 (a) at least one DNA encoding a cellulase which is operably linked to a nucleotide sequence encoding a signal peptide wherein the signal peptide directs the cellulase to an organelle of the transgenic plant; and

15 (b) at least one DNA encoding a ligninase which is operably linked to a nucleotide sequence encoding a signal peptide wherein the signal peptide directs the ligninase to the organelle of the transgenic plant,

wherein the transgenic plant degrades the lignocellulose when ground to produce the plant material.

-2-

5 The transgenic plant of Claim 1 wherein the DNA encoding the cellulase is from an organism selected from the group consisting of *Trichoderma reesei*, *Acidothermus cellulyticus*, *Streptococcus salivarius*, *Actinomyces naeslundii*, and *Thermomonospora fusca*.

-62-

-3-

The transgenic plant of Claim 1 wherein the DNA encoding the cellulase is selected from the group consisting of an *el* gene from *Acidothermus cellulyticus*, a *cbh1* gene from *Trichoderma reesei*, a dextranase gene from *Streptococcus salivarius*, and a beta-glucosidase gene from *Actinomyces naeslundii*.

5

-4-

The transgenic plant of Claim 3 wherein the *el* gene comprises the nucleotide sequence set forth in SEQ ID NO:4, the *cbh1* gene comprises the nucleotide sequence set forth in SEQ ID NO:10, the dextranase gene comprises the nucleotide sequence set forth in SEQ ID NO:8, and the beta-glucosidase gene comprises the nucleotide sequence set forth in SEQ ID NO:6.

5

-5-

The transgenic plant of Claim 1 wherein the DNA encoding the ligninase is from *Phanerochaete chrysosporium*.

-6-

The transgenic plant of Claim 5 wherein the ligninase is *ckg4* comprising the nucleotide sequence set forth in SEQ ID NO:11 or *ckg5* comprising the nucleotide sequence set forth in SEQ ID NO:13.

-7-

The transgenic plant of Claim 1 wherein the DNA encoding the cellulase and the DNA encoding the ligninase are each operably linked to a leaf-specific promoter.

-63-

-8-

The transgenic plant of Claim 7 wherein the leaf-specific promoter is a promoter for *rbcS*.

-9-

The transgenic plant of Claim 1 wherein the nucleotide sequence encoding the signal peptide encodes a signal peptide of *rbcS*.

-10-

The transgenic plant of Claim 8 or 9 wherein the *rbcS* comprises the nucleotide sequence set forth in SEQ ID NO:1.

-11-

The transgenic plant of Claim 1 selected from the group consisting of maize, wheat, barley, rye, hops, hemp, rice, potato, soybean, sorghum, sugarcane, clover, tobacco, alfalfa, arabidopsis, coniferous tree, and deciduous tree.

5

-12-

The transgenic plant of Claim 1 wherein the DNA encoding the cellulase and the DNA encoding the ligninase are stably integrated into nuclear or plastid DNA of the transgenic plant.

-13-

The transgenic plant of Claim 1 wherein transgenic plant further includes a DNA encoding a selectable marker operably linked to a constitutive promoter.

-64-

-14-

The transgenic plant of Claim 13 wherein the DNA encoding the selectable marker provides the transgenic plant with resistance to an antibiotic, an herbicide, or to environmental stress.

-15-

The transgenic plant of Claim 14 wherein the DNA encoding resistance to the herbicide is a DNA encoding phosphinothricin acetyl transferase which confers resistance to the herbicide phosphinothricin.

-16-

The transgenic plant of Claim 1 wherein the organelle of the transgenic plant is selected from the group consisting of nucleus, microbody, endoplasmic reticulum, endosome, vacuole, mitochondria, chloroplast, or plastid.

5

-17-

The transgenic plant of Claim 16 wherein the organelle of the transgenic plant is the chloroplast.

-18-

A transgenic plant which degrades lignins when the transgenic plant is ground to produce a plant material comprising:

at least one DNA encoding a ligninase which is operably linked to a nucleotide sequence encoding a signal peptide wherein the signal peptide directs the ligninase to an organelle of the transgenic plant wherein the transgenic plant degrades the lignins when ground to produce the plant material.

5

-65-

-19-

The transgenic plant of Claim 18 wherein the DNA encoding the ligninase is from *Phanerochaete chrysosporium*.

-20-

The transgenic plant of Claim 19 wherein the ligninase is *ckg4* comprising the nucleotide sequence set forth in SEQ ID NO:11 or *ckg5* comprising the nucleotide sequence set forth in SEQ ID NO:13.

-21-

The transgenic plant of Claim 18 wherein the DNA encoding the ligninase is operably linked to a leaf-specific promoter.

-22-

The transgenic plant of Claim 21 wherein the leaf-specific promoter is a promoter for *rbcS*.

-23-

The transgenic plant of Claim 18 wherein the nucleotide sequence encoding the signal peptide encodes a signal peptide of *rbcS*.

-24-

The transgenic plant of Claim 22 or 23 wherein the *rbcS* comprises the nucleotide sequence set forth in SEQ ID NO:1.

-66-

-25-

The transgenic plant of Claim 18 selected from the group consisting of maize, wheat, barley, rye, hops, hemp, rice, potato, soybean, sorghum, sugarcane, clover, tobacco, alfalfa, arabidopsis, coniferous tree, and deciduous tree.

5

-26-

The transgenic plant of Claim 18 wherein the DNA is stably integrated into nuclear or plastid DNA of the transgenic plant.

-27-

The transgenic plant of Claim 18 wherein the transgenic plant further includes a DNA encoding a selectable marker operably linked to a constitutive promoter.

-28-

The transgenic plant of Claim 27 wherein the DNA encoding the selectable marker provides the transgenic plant with resistance to an antibiotic, an herbicide, or to environmental stress.

-29-

The transgenic plant of Claim 28 wherein the DNA encoding resistance to the herbicide is a DNA encoding phosphinothricin acetyl transferase which confers resistance to the herbicide phosphinothricin.

[illegible]

5

5

The transgenic plant of Claim 18 wherein the organelle of the plant is the chloroplast.

A transgenic plant which degrades cellulose when the transgenic plant is ground to produce a plant material comprising:

5

The transgenic plant of Claim 32 wherein the DNA encoding the cellulase is from an organism selected from the group consisting of *Trichoderma reesei*, *Acidothermus cellulolyticus*, *Streptococcus salivarius*, *Actinomyces naeslundii*, and *Thermomonospora fusca*.

-68-

-34-

5 The transgenic plant of Claim 32 wherein the DNA encoding the cellulase is selected from the group consisting of an *el* gene from *Acidothermus cellulyticus*, a *cbh1* gene from *Trichoderma reesei*, a dextranase gene from *Streptococcus salivarius*, and a beta-glucosidase gene from *Actinomyces naeslundii*.

-35-

5 The transgenic plant of Claim 34 wherein the *el* gene comprises the nucleotide sequence set forth in SEQ ID NO:4, the *cbh1* gene comprises the nucleotide sequence set forth in SEQ ID NO:10, the dextranase gene comprises the nucleotide sequence set forth in SEQ ID NO:8, and the beta-glucosidase gene comprises the nucleotide sequence set forth in SEQ ID NO:6.

-36-

The transgenic plant of Claim 32 wherein DNA encoding the cellulase is operably linked to a leaf-specific promoter.

-37-

The transgenic plant of Claim 36 wherein the leaf-specific promoter is a promoter for *rbcS*.

-38-

The transgenic plant of Claim 32 wherein the nucleotide sequence encoding the signal peptide encodes a signal peptide of *rbcS*.

-39-

The transgenic plant of Claim 37 or 38 wherein the *rbcS* comprises the nucleotide sequence set forth in SEQ ID NO:1.

-69-

-40-

5

The transgenic plant of Claim 32 selected from the group consisting of maize, wheat, barley, rye, hops, hemp, rice, potato, soybean, sorghum, sugarcane, clover, tobacco, alfalfa, arabidopsis, coniferous tree, and deciduous tree.

-41-

The transgenic plant of Claim 32 wherein the DNA is stably integrated into nuclear or plastid DNA of the transgenic plant.

-42-

The transgenic plant of Claim 32 wherein the transgenic plant further includes a DNA encoding a selectable marker operably linked to a constitutive promoter.

-43-

The transgenic plant of Claim 42 wherein the DNA encoding the selectable marker provides the transgenic plant with resistance to an antibiotic, an herbicide, or to environmental stress.

-44-

The transgenic plant of Claim 43 wherein the DNA encoding resistance to the herbicide is a DNA encoding phosphinothricin acetyl transferase which confers resistance to the herbicide phosphinothricin.

-70-

-45-

5 The transgenic plant of Claim 32 wherein the organelle of the transgenic plant is selected from the group consisting of nucleus, microbody, endoplasmic reticulum, endosome, vacuole, mitochondria, chloroplast, or plastid.

-46-

The transgenic plant of Claim 45 wherein the organelle of the transgenic plant is the chloroplast.

-47-

A method for producing a transgenic plant which degrades lignocellulose when the transgenic plant is ground to produce a plant material comprising:

5 (a) providing a first transgenic plant which includes a DNA encoding a cellulase which is operably linked to a nucleotide sequence encoding a signal peptide wherein the signal peptide directs the cellulase to an organelle of the transgenic plant and a second transgenic plant which includes a DNA encoding a ligninase which is operably linked to a nucleotide sequence encoding a signal peptide wherein the signal peptide directs the ligninase to the organelle of the transgenic plant; and

10 (b) mating by sexual fertilization the first and the second transgenic plants to produce a third transgenic plant which includes the first DNA encoding the cellulase and the second DNA encoding the ligninase, wherein the transgenic plant degrades the lignocellulose when ground to produce the plant material.

15

20

-71-

-48-

5 The method of Claim 47 wherein the DNA encoding the cellulase is from an organism selected from the group consisting of *Trichoderma reesei*, *Acidothermus cellulyticus*, *Streptococcus salivarius*, *Actinomyces naeslundii*, and *Thermomonospora fusca*.

-49-

5 The method of Claim 47 wherein the DNA encoding the cellulase is selected from the group consisting of an *el* gene from *Acidothermus cellulyticus*, a *cbh1* gene from *Trichoderma reesei*, a dextranase gene from *Streptococcus salivarius*, and a beta-glucosidase gene from *Actinomyces naeslundii*.

-50-

5 The method of Claim 49 wherein the *el* gene comprises the nucleotide sequence set forth in SEQ ID NO:4, the *cbh1* gene comprises the nucleotide sequence set forth in SEQ ID NO:10, the dextranase gene comprises the nucleotide sequence set forth in SEQ ID NO:8, and the beta-glucosidase gene comprises the nucleotide sequence set forth in SEQ ID NO:6.

-51-

The method of Claim 47 wherein the DNA encoding the ligninase is from *Phanerochaete chrysosporium*.

-52-

The method of Claim 51 wherein the ligninase is *ckg4* comprising the nucleotide sequence set forth in SEQ ID NO:11 or *ckg5* comprising the nucleotide sequence set forth in SEQ ID NO:13.

-72-

-53-

The method of Claim 47 wherein the DNA encoding the cellulase and the DNA encoding the ligninase are each operably linked to a leaf-specific promoter such as a promoter for *rbcS*.

-54-

The method of Claim 53 wherein the leaf-specific promoter is a promoter for *rbcS*.

-55-

The method of Claim 47 wherein the nucleotide sequence encoding the signal peptide encodes a signal peptide of *rbcS*.

-56-

The method of Claim 54 or 55 wherein the *rbcS* comprises the nucleotide sequence set forth in SEQ ID NO:1.

-57-

The method of Claim 47 selected from the group consisting of maize, wheat, barley, rye, hops, hemp, rice, potato, soybean, sorghum, sugarcane, clover, tobacco, alfalfa, arabidopsis, coniferous tree, and deciduous tree.

-58-

The method of Claim 47 wherein the DNA encoding the cellulase and the DNA encoding the ligninase are stably integrated into nuclear or plastid DNA of the transgenic plant.

-73-

-59-

The method of Claim 47 wherein the first, second, or both transgenic plants further includes a DNA encoding a selectable marker operably linked to a constitutive promoter.

-60-

The method of Claim 59 wherein the DNA encoding the selectable marker provides the transgenic plant with resistance to an antibiotic, an herbicide, or to environmental stress.

-61-

The method of Claim 60 wherein the DNA encoding resistance to the herbicide is a DNA encoding phosphinothricin acetyl transferase which confers resistance to the herbicide phosphinothricin.

-62-

The method of Claim 47 wherein the organelle of the transgenic plant is selected from the group consisting of nucleus, microbody, endoplasmic reticulum, endosome, vacuole, mitochondria, chloroplast, or plastid.

-63-

The method of Claim 62 wherein the organelle of the transgenic plant is the chloroplast.

-74-

-64-

The method of Claim 47 wherein progeny of the third transgenic plant are mated by sexual fertilization to a transgenic plant selected from the group consisting of the first, second, and third transgenic plants to produce a transgenic plant comprising multiples of genes encoding cellulases and ligninases.

11/11/2003 11:11:11 AM

A method for converting lignocellulose in a plant material to fermentable sugars comprising:

5 (a) providing a transgenic plant which includes at least one DNA encoding a cellulase which is operably linked to a nucleotide sequence encoding a signal peptide wherein the signal peptide directs the cellulase to an organelle of the transgenic plant and a
10 at least one DNA encoding a ligninase which is operably linked to a nucleotide sequence encoding a signal peptide wherein the signal peptide directs the ligninase to the organelle of the transgenic plant;

15 (b) growing the transgenic plant for a time sufficient for the transgenic plant to accumulate a sufficient amount of the cellulase and the ligninase in the organelle of the transgenic plant;

(c) harvesting the transgenic plant which has accumulated the cellulase and ligninase in the organelle of the transgenic plant;

20 (d) grinding the transgenic plant for a time sufficient to produce the plant material wherein the cellulase and ligninase produced by the transgenic plant are released from the organelle of the transgenic plant;

25 (e) incubating the plant material for a time sufficient for the cellulase and ligninase in the plant material to produce the fermentable sugars from the lignocellulose in the plant material; and

(f) extracting the fermentable sugars produced from the lignocellulose by the cellulase and the ligninase from the plant material.

-76-

-66-

The method of Claim 65 wherein the DNA encoding the cellulase is from an organism selected from the group consisting of *Trichoderma reesei*, *Acidothermus cellulyticus*, *Streptococcus salivarius*, *Actinomyces naeslundii*, and *Thermomonospora fusca*.

-67-

The method of Claim 65 wherein the DNA encoding the cellulase is selected from the group consisting of an *el* gene from *Acidothermus cellulyticus*, a *cbh1* gene from *Trichoderma reesei*, a dextranase gene from *Streptococcus salivarius*, and a beta-glucosidase gene from *Actinomyces naeslundii*.

-68-

The method of Claim 67 wherein the *el* gene comprises the nucleotide sequence set forth in SEQ ID NO:4, the *cbh1* gene comprises the nucleotide sequence set forth in SEQ ID NO:10, the dextranase gene comprises the nucleotide sequence set forth in SEQ ID NO:8, and the beta-glucosidase gene comprises the nucleotide sequence set forth in SEQ ID NO:6.

-69-

The method of Claim 65 wherein the DNA encoding the ligninase is from *Phanerochaete chrysosporium*.

-70-

The method of Claim 69 wherein the ligninase is *ckg4* comprising the nucleotide sequence set forth in SEQ ID NO:11 or *ckg5* comprising the nucleotide sequence set forth in SEQ ID NO:13.

-77-

-71-

The method of Claim 65 wherein DNA encoding the cellulase and the DNA encoding the ligninase are each operably linked to a leaf-specific promoter.

-72-

The transgenic plant of Claim 71 wherein the leaf-specific promoter is a promoter for *rbcS*.

-73-

The method of Claim 65 wherein the nucleotide sequence encoding the signal peptide encodes a signal peptide of *rbcS*.

-74-

The method of Claim 72 or 73 wherein the *rbcS* comprises the nucleotide sequence set forth in SEQ ID NO:1.

-75-

The method of Claim 65 selected from the group consisting of maize, wheat, barley, rye, hops, hemp, rice, potato, soybean, sorghum, sugarcane, clover, tobacco, alfalfa, arabidopsis, coniferous tree, and deciduous tree.

-76-

The method of Claim 65 wherein the first and second DNAs are stably integrated into nuclear or plastid DNA of the transgenic plant.

-78-

-77-

The method of Claim 65 wherein transgenic plant further includes a DNA encoding a selectable marker operably linked to a constitutive promoter.

-78-

The method of Claim 77 wherein the DNA encoding the selectable marker provides the transgenic plant with resistance to an antibiotic, an herbicide, or to environmental stress.

-79-

The method of Claim 78 wherein the DNA encoding resistance to the herbicide is a DNA encoding phosphinothricin acetyl transferase which confers resistance to the herbicide phosphinothricin.

-80-

The method of Claim 65 wherein the organelle of the transgenic plant is selected from the group consisting of nucleus, microbody, endoplasmic reticulum, endosome, vacuole, mitochondria, chloroplast, or plastid.

-81-

The method of Claim 80 wherein the organelle of the transgenic plant is the chloroplast.

-82-

The method of Claim 65 wherein the plant material further includes a plant material made from a non-transgenic plant.

A method for converting lignocellulose in a plant material to fermentable sugars comprising:

(a) providing a transgenic plant which includes at least one DNA encoding a cellulase which is operably linked to a nucleotide sequence encoding a signal peptide wherein the signal peptide directs the cellulase to an organelle of the transgenic plant;

(b) growing the transgenic plant for a time sufficient for the transgenic plant to accumulate a sufficient amount of the cellulase in the organelle of the transgenic plant;

(c) harvesting the transgenic plant which has accumulated the cellulase in the organelle of the transgenic plant;

(d) grinding the transgenic plant for a time sufficient to produce a plant material wherein the cellulase is released from the organelle in the transgenic plant;

(e) mixing the plant material with a fungus that produces a ligninase;

(f) incubating the transgenic plant material with the fungus for a time sufficient for the cellulase released from the transgenic plant and the ligninase provided by the fungus to degrade the lignocellulose in the plant material to produce the fermentable sugars; and

(g) extracting the fermentable sugars produced from the lignocellulose in the plant material.

-80-

-84-

The method of Claim 83 wherein the DNA encoding the cellulase is from an organism selected from the group consisting of *Trichoderma reesei*, *Acidothermus cellulyticus*, *Streptococcus salivarius*, *Actinomyces naeslundii*, and *Thermomonospora fusca*.

5

-85-

The method of Claim 83 wherein the DNA encoding the cellulase is selected from the group consisting of an *e1* gene from *Acidothermus cellulyticus*, a *cbh1* gene from *Trichoderma reesei*, a dextranase gene from *Streptococcus salivarius*, and a beta-glucosidase gene from *Actinomyces naeslundii*.

5

-86-

The method of Claim 85 wherein the *e1* gene comprises the nucleotide sequence set forth in SEQ ID NO:4, the *cbh1* gene comprises the nucleotide sequence set forth in SEQ ID NO:10, the dextranase gene comprises the nucleotide sequence set forth in SEQ ID NO:8, and the beta-glucosidase gene comprises the nucleotide sequence set forth in SEQ ID NO:6.

5

-87-

The method of Claim 83 wherein the DNA encoding the cellulase is operably linked to a leaf-specific promoter.

-88-

The method of Claim 87 wherein the leaf-specific promoter is a promoter for *rbcS*.

-81-

-89-

The method of Claim 83 wherein the nucleotide sequence encoding the signal peptide encodes a signal peptide of *rbcS*.

-90-

The method of Claim 88 or 89 wherein the *rbcS* comprises the nucleotide sequence set forth in SEQ ID NO:1.

-91-

The method of Claim 83 selected from the group consisting of maize, wheat, barley, rye, hops, hemp, rice, potato, soybean, sorghum, sugarcane, clover, tobacco, alfalfa, arabidopsis, coniferous tree, and deciduous tree.

5

-92-

The method of Claim 83 wherein the DNA is stably integrated into nuclear or plastid DNA of the transgenic plant.

-93-

The method of Claim 83 wherein the transgenic plant further includes a DNA encoding a selectable marker operably linked to a constitutive promoter.

-94-

The method of Claim 93 wherein the DNA encoding the selectable marker provides the transgenic plant with resistance to an antibiotic, an herbicide, or to environmental stress.

-82-

-95-

The method of Claim 94 wherein the DNA encoding resistance to the herbicide is a DNA encoding phosphinothricin acetyl transferase which confers resistance to the herbicide phosphinothricin.

-96-

The method of Claim 83 wherein the organelle of the transgenic plant is selected from the group consisting of nucleus, microbody, endoplasmic reticulum, endosome, vacuole, mitochondria, chloroplast, or plastid.

5

-97-

The method of Claim 96 wherein the organelle of the transgenic plant is the chloroplast.

-98-

The method of Claim 83 wherein the fungus is *Phanerochaete chrysosporium*.

-99-

The method of Claim 83 wherein the plant material further includes a plant material made from a non-transgenic plant.

-100-

The transgenic plant of Claim 1 wherein the lignocellulose is degraded to fermentable sugars.

-101-

The transgenic plant of Claim 32 wherein the cellulose is degraded to fermentable sugars.

